The 16S rDNA metagenetic monitoring of refrigerated food products for understanding the kinetics of microbial subpopulations at different storage temperatures: the example of white pudding

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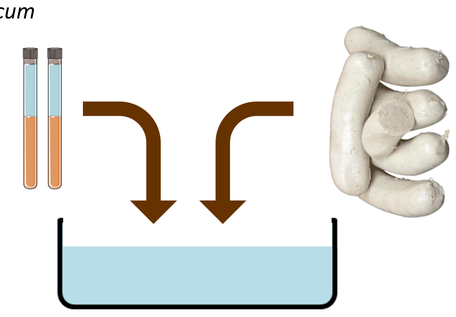
Introduction

For a clear understanding of the mechanisms that lead to the spoilage of food products, the classical microbiology is not sufficient enough. Fortunately, molecular technologies (like high throughput sequencing methods) can elucidate the microbial communities, including the identification and quantification of culturable and non-culturable organisms, at a much higher resolution than was previously possible with culture-based methods. The present work proposes to follow the evolution of the main microflora's components in white pudding, a typical Belgian meat product.

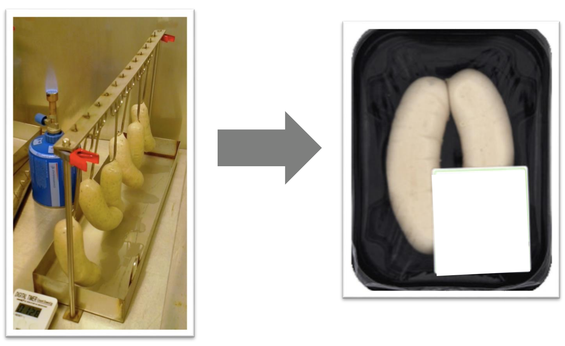
Material and methods

Inoculated strains :

- *Carnobacterium maltaromaticum*
- *Lactobacillus fuchuensis*
- *Lactobacillus graminis*
- *Lactobacillus oligofermentans*
- *Lactococcus lactis*
- *Leuconostoc mesenteroides*
- *Raoultella terrigena*
- *Serratia* sp.

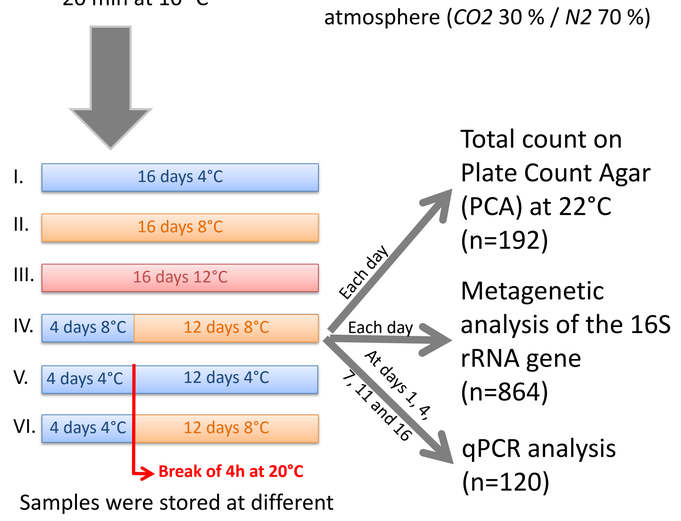


The white-pudding were soaked 2 min in a bath of sterile water containing a mix of the eight strains at the same concentration. The objective is to reach a global concentration of 3 ± 1 log *CFU.g*$^{-1}$ on the product



Drying step of 20 min at 10 °C

Two white puddings were packed in a tray (PP/EVOH/PP) under modified atmosphere (*CO2* 30 % / *N2* 70 %)



Samples were stored at different temperature, constant or dynamic : I. 4°C; II. 8°C; III. 12°C; IV. 4-8°C; V. 4/20-4°C; VI. 4/20-8°V

Results

A combination was made between the PCA results of the microflora at 22 °C and the relative proportions of strains given by metagenetic in order to obtain estimate counts for the strains. These data were used to obtain growth parameters for each strains and temperature conditions tested.

Growth parameters obtained for the constant temperature conditions

4 °C	Nmax[a]	Stationary phase[b]	µmax[c]	Class[d]
C. maltaromaticum	8.6	12	0.07	D
Lb. fuchuensis	8.5	16	0.05	S
Lb. graminis	7.6	16	0.03	S
Ln. mesenteroides	8.1	16	0.03	S
Lc. lactis	4.9	12	0.05	I
Serratia sp.	6.7	12	0.04	I

8 °C	Nmax[a]	Stationary phase[b]	µ max[c]	Class[d]
C. maltaromaticum	8.1	8	0.10	D
Lc. lactis	8.4	10	0.09	S
Lb. fuchuensis	8.3	10	0.09	S
Ln. mesenteroides	8.9	10	0.10	S
Lb. graminis	7.6	8	0.08	I
Serratia sp.	6.7	8	0.10	I

8 °C	Nmax[a]	Stationary phase[b]	µ max[c]	Class[d]
C. maltaromaticum	8.1	8	0.10	D
Lc. lactis	8.4	10	0.09	S
Lb. fuchuensis	8.3	10	0.09	S
Ln. mesenteroides	8.9	10	0.10	S
Lb. graminis	7.6	8	0.08	I
Serratia sp.	6.7	8	0.10	I

a: bacterial concentration at day 16 (Nmax, log CFU.g^{-1}); b: time to reach the stationary phase (days); c: maximal bacterial growth rate (µmax, *h-1*).

Results allowed the bacterial strain subdivision into three classes (d).

- D (dominant): the highest growth rate (µmax), a maximal concentration (Nmax) between 8 and 9 log *CFU.g*$^{-1}$, and a stationary phase rapidly reached.
- I (inhibited): lesser or equal growth rate than D but an inferior Nmax value and a growth stopped on the same time that the D species.
- S (subdominant): all other bacterial species that continued to growth when the D organisms reached the stationary phase, with a growth rate generally lesser but a high maximal concentration.

Comparison of growth rates during exponential phase for the dynamic temperature conditions using ANOVA-test

	4°C VS. 4-8°C	4°C VS. 4/20-4°C	4-8°C VS. 4/20-8°C
C. maltaromaticum	4-8>4**	Ø	Ø
Lc. lactis	4-8>4**	NA	Ø
Lb. fuchuensis	4-8>4**	Ø	Ø
Lb. graminis	4-8>4**	Ø	Ø
Ln. mesenteroides	4-8>4***	4/20-4>4*	Ø
Serratia sp.	4-8>4**	Ø	Ø

Ø: no significant statistical difference; *: significant statistical difference, p-value < 0.05; **: high significant statistical difference, p-value < 0.01; ***: highly significant statistical difference, p-value < 0.001; >: superior value; NA: not avalaibale.

Conclusion

The data obtained show different groups inside the ecosystem, interacting the ones with the others, illustrating the Jameson effect (the inhibited vs. the dominant), or not (the subdominant vs. the dominant). Considering the dynamic temperature conditions of storage, these issues show that a no respect of the good storage temperatures is more prejudicial than a break of a few hour at room temperature. Further studies will focus on a deeper understanding of the interaction between the different group of bacterial species highlighted in this work.